

CLAIMS

1. A yeast strain lacking ethanol production ability or having a reduced ethanol production ability with respect
5 to wild-type yeasts of the same strains and transformed with at least one copy of a gene coding for lactic dehydrogenase, functionally linked to promoter sequences allowing the expression of said gene in yeasts.
2. A yeast strain according to claim 1 having an
10 ethanol production ability which is less than about 60% compared to wild type yeasts of the same strain.
3. A yeast strain according to claim 1 or 2 having a reduced pyruvate decarboxylase and/or pyruvate dehydrogenase activities and at least one copy of a
15 nucleic acid sequence encoding for lactic dehydrogenase protein.
4. A yeast strain according to claim 3 having a reduced pyruvate dehydrogenase activity.
5. A yeast strain according to claim 3 having a reduced
20 pyruvate decarboxylase activity.
6. A yeast strain according to claim 3 having both a reduced pyruvate dehydrogenase activity and a reduced pyruvate decarboxylase activity.
7. A yeast strain according to any one of claims 1-6
25 wherein the gene or the genes coding for pyruvate decarboxylase, for pyruvate dehydrogenase or both has or have been disrupted by deletion or insertion by means of selectable marker(s).
8. A yeast strain according to claim 7, wherein the
30 selectable marker is a URA3 marker.
9. A yeast strain according to claim 8 wherein the

20060220 "E" 020602

selectable marker is the URA3 marker from Saccharomyces cerevisiae.

10. A yeast strain according to claim 7, wherein the
5 resistance to toxic compounds.

11. A yeast strain according to any one of claims 1-10,
selected from Saccharomyces, Kluyveromyces, Torulaspora
and Zygosaccharomyces species.

12. A yeast strain according to claim 11 which is a
10 strain of Saccharomices cerevisise.

13. A yeast strain according to claim 11 which is a
strain of Kluyveromyces lactis.

14. A yeast strain according to claim 11 which is a
strain of Torulaspora delbrueckii.

15 15. A yeast strain according to claim 11 which is a
strain of Zygosaccharomyces bailii.

16. A yeast strain selected from Kluyveromyces,
Torulaspora and Zygosaccharomyces species transformed
with at least one copy of a gene coding for lactic
20 dehydrogenase, functionally linked to promoter sequences
allowing the expression of said gene in said yeasts.

17. A yeast strain according to any one of claims 1 to
16 transformed with a gene coding for the bovine lactate
dehydrogenase.

25 18. A yeast strain according to any one of claims 1 to
16 transformed with a gene coding for a bacterial
lactate dehydrogenase.

19. A yeast strain according to any one of claims 1 to
17 in which the gene coding for lactate dehydrogenase is
30 integrated in the yeast genome.

20. A yeast strain according to any one of claims 1-19

20060220 "020602"

which has been transformed by an expression vector comprising a promoter sequence and a DNA sequence coding for lactate dehydrogenase under the regulation of said promoter sequence.

5 21. A yeast strain according to claim 20 wherein the promoter sequence is a pyruvate decarboxylase gene promoter.

22. A yeast strain according to claim 21 wherein the promoter sequence is a Kluyveromyces pyruvate
10 decarboxylase gene promoter.

23. A yeast strain according to any one of the preceding claims, overexpressing a lactate transporter.

24. A yeast strain according to claim 23, in which the lactate transporter is JEN1.

15 25. A vector comprising a DNA sequence coding for a lactic dehydrogenase functionally linked to a pyruvate decarboxylase gene promoter.

26. A vector according to claim 25, wherein the promoter sequence is a Kluyveromyces pyruvate decarboxylase gene
20 promoter.

27. A vector according to claim 26, wherein the promoter sequence is a Kluyveromyces lactis pyruvate decarboxylase gene promoter.

28. A process for the preparation of lactic acid
25 comprising the growth of a recombinant yeast strain of claims 1-24 in a fermentation medium containing a carbon source and the recovery of lactic acid from the fermentation medium.

29. A process according to claim 28, wherein the carbon
30 source is selected from one or more of glucose, fructose, galactose, lactose, sucrose, raffinose, maltose,

cellobiose, arabinose, xylose.

30. A process for the preparation of lactic acid according to claim 28 or 29, wherein the fermentation medium contains less than 5 mM of Mg^{++} and/or less than
5 0.02 mM of Zn^{++} .

31. A process according to claims 28-30, wherein the fermentation medium has a pH of 7 or less.

32. A process according to claim 31 wherein the pH is 4.5 or less.

10 33. A process according to claim 32 wherein the pH is 3 or less.

34. A process according to claim 28 for the preparation of D- or L- lactic acid or a mixture of the two.

20060220 13:00:00